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INBREEDING AND REPRODUCTIVE INVESTMENT IN THE ANT *FORMICA EXSECTA*

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In social animals, inbreeding depression may manifest by compromising care or resources individuals receive from inbred group members. We studied the effects of worker inbreeding on colony productivity and investment in the ant *Formica exsecta*. The production of biomass decreased with increasing inbreeding, as did biomass produced per worker. Inbred colonies produced fewer gynes (unmated reproductive females), whereas the numbers of males remained unchanged. As a result, sex ratios showed increased male bias, and the fraction of workers increased among the diploid brood. Males raised in inbred colonies were smaller, whereas the weight of gynes remained unchanged. The results probably reflect a trade-off between number and quality of offspring, which is expected if the reproductive success of gynes is more dependent on their weight or condition than it is for males. As males are haploid (with the exception of abnormal diploid males produced in very low frequencies in this population), and therefore cannot be inbred themselves, the effect on their size must be mediated through the workers of the colony. We suggest the effects are caused by the inbred workers being less proficient in feeding the growing larvae. This represents a new kind of social inbreeding depression that may affect sex ratios as well as caste fate in social insects.

KEY WORDS: **Inbreeding, reproduction, resource allocation, sex ratio, social insects, trade-off.**

Human-induced environmental change eradicates populations across all taxa, decreasing population sizes and reducing their connectivity. As a result, inbreeding, with loss of genetic diversity in its wake, may become more common, further threatening the well being and survival of populations (Lande 1988; Frankham 1995a; Spielman et al. 2004). An increase in homozygosity due to inbreeding has deleterious consequences, inbreeding depression, both at the individual and population level (Wright 1977; Charlesworth and Charlesworth 1987; Frankham 1995b; Lynch and Walsh 1998; Crnokrak and Roff 1999; Hedrick and Kalinowski 2000; Keller and Waller 2002). Inbreeding depression is typically greatest in fitness-related traits, such as fertility, egg hatching rate, and neonatal survival (DeRose and Roff 1999), but inbred individuals may also be more susceptible to environmental stress (Bijlsma et al. 1999; Keller and Waller 2002; Reed et al. 2002; Bijlsma and Loeschcke 2005). Recent studies on the fruit fly suggest that at the level of the genome, inbreeding depression may present a form of genetic stress that changes the regulation of genes involved in metabolism, defense, and stress responses, thus decreasing the energy efficiency of inbred individuals (Kristensen 2006; Ayroles et al. 2009). Consistent with this view, inbreeding reduces growth rate (Roff 1998), increases resting metabolic rate in crickets (Ketola and Kotiaho 2009), and reduces mass gain in the White-footed mouse (Jimenez et al. 1994). This raises the question whether inbreeding affects the way individuals allocate resources between growth and reproduction. This question is particularly pertinent in eusocial animals, where owing to division of labor, inbreeding depression may manifest through both the individuals themselves but also through compromised energy allocation and care by other colony members.

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To date, most of our knowledge on the effects of inbreeding in wild populations comes from a few vertebrate systems (e.g., the song sparrow, Keller 1998; the Soay sheep, Coltman et al. 1999; the red deer, Coulson et al. 1999; the reed warbler, Hansson et al. 2002), and only a handful of studies have assessed the direct effects of genetic diversity in natural populations of arthropods in general (e.g., the Glanville fritillary *Melitaea cinxia*, Saccheri et al. 1998; the wolf spiders *Rabidosa punctuata* and *Rabidosa rabida*, Reed et al. 2007) or social insects in particular (the Japanese bumblebee *Bombus florilegus*, Takahashi et al. 2008, and the narrow-headed ant *Formica exsecta,* Haag-Liautard et al. 2009).

Social insects are characterized by reproductive division of labor between the female castes, queens, and workers, which is considered the key to their ecological success (Hölldobler and Wilson 1990). However, owing to caste specialization, social insects may be especially susceptible to inbreeding and loss of genetic diversity, as populations often comprise relatively few reproductive individuals and therefore have a small effective population size (Chapman and Bourke 2001; Packer and Owen 2001). Inbreeding has important implications on social Hymenoptera also because they are haplodiploid, with males arising from unfertilized eggs and diploid females developing to either gynes (unmated reproductive females) or workers. Under inbreeding, the complementary sex determination mechanism of Hymenoptera (Cook 1993; Beye et al. 2003) results in infertile diploid males being produced at a fitness cost, both at the level of the population (Stouthamer et al. 1992; Zayed et al. 2004; Zayed and Packer 2005; Mäki-Petäys and Breen 2006; Takahashi et al. 2008) and the colony (Ross and Fletcher 1986; Duchateau et al. 1994; Pamilo et al. 1994; Tarpy and Page 2002, but see Cowan and Stahlhut 2004). This sex-determining mechanism may also result in more male-biased sex ratios as fertilized female-destined eggs, homozygous at the sex-determining locus, attain a male phenotype. Conversely, male haploidy may promote the purging of deleterious recessive alleles, potentially clearing populations of genetic load and diminishing the harmful effects of inbreeding (Charlesworth and Charlesworth 1987, 1999). This does not, however, apply to traits with caste- or sex-specific expression (Saito et al. 2000; Graff et al. 2007). Consistent with this, an earlier study showed that inbreeding reduced the longevity of ant queens (Haag-Liautard et al. 2009).

In social insects, inbreeding may occur at two levels, independent of each other: the reproductive queen may be inbred and/or the worker force may be inbred if the queen has mated with a related male (Haag-Liautard et al. 2009). In the latter case, inbreeding may impact colony performance if it compromises worker efficiency and so undermines the colony's ability to gain resources. Changes in resource levels may, in turn, impact the way these are allocated between colony maintenance and reproduction, induce changes in sex-allocation ratios (apart from the production of diploid males), and/or a reduction in the number and size of the reproductives produced (Smith and Fretwell 1974). Inbreeding may also have direct effects on caste determination. Although caste determination in most ants is mainly environmentally controlled (Wheeler 1986, reviewed in Evans and Wheeler 2001, but see Julian et al. 2002; Volny and Gordon 2002), inbred diploid larvae may be more inclined to enter the worker developmental pathway if caste determination is contingent on the growth rate of individual larvae and inbreeding affects growth rate (Roff 1998). The underlying mechanism could be either intrinsic, if the heterozygosity of the larva itself affects its growth rate, or extrinsic, if the curtailed growth results from reduced feeding or care provided by inbred workers. Indeed, the effects of homozygosity on caste determination have been proposed as a mechanism explaining the differences in inbreeding coefficients between queens and workers of several *Formica* species (Sundström 1993; Sundström et al. 2003; Hannonen et al. 2004, but see Haag-Liautard et al. 2009).

In an earlier study on the narrow-headed ant *F. exsecta,* we have shown that the higher the level of inbreeding in brood-tending workers, the lower the numerical proportion of reproductives (Haag-Liautard et al. 2009). This begs the question whether inbreeding also influences sex ratios and patterns of investment in reproductive females and males. As in solitary organisms, trade-offs between the size and number of offspring may differ for male and female reproductives. Thus, following the argument by Smith and Fretwell (1974), if the reproductive success of offspring is contingent on a minimum size in one sex and inbreeding reduces colony productivity, leading to a trade-off between the size and number of offspring, fewer rather than smaller individuals of that sex will be produced by colonies with inbred workers. If the constraints on offspring size differ between the sexes, numerical sex ratios may shift toward the less-demanding sex. Given that colony foundation, and thus the reproductive success of young queens in monogyne (single queen per colony) species of ants is contingent on sufficient nutritional reserves, we hypothesize that female reproductives in particular may be size-constrained (Nonacs 1986; Keller and Passera 1989; Sundström 1995; Rosenheim et al. 1996). Males are short-lived and their mating success less likely to depend on their size (Boomsma et al. 2005). Hence, we expect inbred colonies to produce a male-biased sex ratio, and that, owing to compromised worker performance, a higher proportion of the diploid eggs will develop into workers rather than gynes.

Here, we tested whether worker inbreeding affects colony performance, and sex allocation in the two sexes in the narrow-headed ant *F. exsecta*. We used sex ratios, size, and weight of female and male reproductives, and caste ratios in diploid brood as proxies for colony performance, and compare these

parameters for colonies with different degrees of worker inbreeding. The adult workers are sisters to the brood produced, thus their level of inbreeding should be equal to that of all the diploid offspring (gynes and young workers) produced in the colonies. In addition, we tested whether inbreeding directly affects caste ratios among diploid, female-destined brood by comparing the level of inbreeding between contemporary gyne and worker brood within colonies, and by comparing female caste ratios across colonies with varying degree of inbreeding.

Material and Methods

STUDY POPULATION

Our study population of the ant *F. exsecta* is located on five islands close to the Tvärminne Zoological station in Hanko, on the SW coast of Finland, and has been surveyed since 1994 (Sundström et al. 1996, 2003; Chapuisat et al. 1997; Haag-Liautard et al. 2009; Vitikainen and Sundström 2011). The population currently comprises approximately 100 colonies, the majority of which are headed by a single reproductive queen (monogyny), which is either singly or multiply mated. New reproductive brood is produced annually, intermixed with worker brood. The reproductives depart on a nuptial flight, after which the young queens shed their wings and found new colonies. A considerable fraction of the colonies have been genotyped repeatedly, and we have found no evidence for queen supersedure or changes in numbers of patrilines even at time intervals spanning 15 years. Once established, the colonies persist in the same location for decades, only rarely relocating (Pamilo 1991; L. Sundström, pers. obs.). Hence, the genetic composition of the worker force remains unchanged for the life span of the colony. For this study, data were collected from 56 monogyne colonies that produced reproductives in 2008.

MEASURING INBREEDING

A minimum of 10 workers per colony had previously been genotyped at 10 microsatellite loci (Haag-Liautard et al. 2009). The degree of inbreeding (HL) among workers was estimated as the average homozygosity per locus weighted by the information content of the locus in question (Aparicio et al. 2006). For comparison of methods, we also calculated the expected inbreeding coefficients among the workers, using the relatedness between queen and her mate(s), as described in Liautard and Sundström (2005). For this, the genotypes of colony queens and fathers were deduced from worker and male offspring assuming a minimum number of patrilines (Sundström et al. 1996). In addition, to test whether inbreeding affects caste determination within single cohorts of female brood through random segregation of genes, we estimated the degree of inbreeding (HL) separately for young workers and gynes produced in the same cohort of pupae from 15 colonies in 2005, nine of which were also sampled for productivity in 2008. These estimates were based on 15–22 workers and 15–22 new queens per colony, genotyped at nine of the 10 loci used in Haag-Liautard et al. (2009); Fe17 was omitted due to amplification problems.

Concerns are occasionally raised whether inbreeding estimates based on 10 loci reflect genome-wide inbreeding and so are justified (David 1998; Balloux et al. 2004; Markert et al. 2004; Slate et al. 2004). However, the structuring and small size of our study population and the high variation in homozygosity between colonies (Haag-Liautard et al. 2009) increases the likelihood of detecting inbreeding effects even with as few as 10 loci (Balloux et al. 2004; Hansson and Westerberg 2008). Furthermore, in small inbred populations, variation in the inbreeding coefficients of individuals reflects the pedigree structure of the population and is the most likely explanation for associations between homozygosity at marker loci and fitness traits (Bierne et al. 2000; Szulkin et al. 2010).

COLONY SIZE AND PRODUCTIVITY

We determined colony size by mark–recapture of overwintered (old) workers in May–June 2008, before the emergence of new brood. On average 410 ± 47 (SE) workers per colony were marked with spray paint (Maston AutoMix Silver, Veikkola, Finland), and a subsample of 60 workers was collected two days after marking. Colony size was calculated with a correction for small recapture samples following Pollock et al. (1990) as $[(x + 1) + (y + 1)/(z + 1)] - 1$, where x equals the total number of captured individuals, y equals the number of marked individuals, and z equals the number of unmarked individuals in the sample. Similarly, we measured colony productivity by mark–recapture of pupae in June–July 2008 before adults had emerged. The first cohort of brood comprises the entire sexual production of the colony in that season, as well as part of the new workers. On average 334 ± 29 (SE) pupae per colony were marked with a permanent marker (Artline 700, Shachihata, Japan), and immediately returned to the colony. After five days, a sample of 50–60 pupae per colony was collected to estimate productivity as above, and determine the proportion of worker brood and the sex ratio among the reproductives.

INVESTMENT IN REPRODUCTIVES

To determine weight at emergence and maturity, we collected newly emerged reproductives (callows) as well as reproductives at the point of departure on nuptial flights from the same colonies as above. The first sample comprised 2–10 newly emerged males (5 ± 0.45, mean $\pm$ SE) and gynes (8 ± 0.78, mean $\pm$ SE) from within the nest, and the second 4–10 individuals of each sex from the surface of the nest when they were about to take off for their nuptial flight (males: 8 ± 0.55, gynes 7 ± 0.73, mean $\pm$ SE). The interval between the two collection events was approximately 10 days for males and approximately three weeks for gynes.

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The individuals were weighed to the nearest 0.1 mg on a Mettler AB304-S balance, after drying to a constant weight at 80°C for approximately 4 h. The average weight gain per colony was then calculated as the difference in average weight between the two samples.

We used head width as a measure of the overall size of the individuals, as this is a standard morphometric in ants (Deslippe and Savolainen 1994), and head size predicts individual size across castes in *F. Exsecta* (Fortelius et al. 1987). The heads were severed from the body and photographed under a Leica MZ10F stereomicroscope, with a Leica DFL490 camera attached to the microscope. The head width of each individual was measured to the nearest micrometers using Image J (available at http://rsb.info.nih.gov/ij; developed by Wayne Rasband, National Institutes of Health, Bethesda, MD).

STATISTICAL ANALYSES

We tested the effect of colony and maturation stage (callow vs. mature) on the size of reproductives using a nested ANOVA, with colonies nested within maturation stage, to account for possible changes in offspring size between the two sampling events. Given that colonies regularly produce single-sex reproductive broods, we could not obtain samples of both sexes from all colonies. Hence, we analyzed female and male data separately. For comparisons between callow and mature reproductives, we included only colonies where a sample of both maturation stages was available, whereas all individuals were included in the colony-specific average weight and size measures. All measures are given as the mean ± the standard error.

We calculated the colony-specific investment in reproductives by multiplying the number of males and females produced in each colony by their respective colony-specific average weights at maturity. Although the species is monomorphic, with no distinct morphological worker castes, the size of the workers varies among the colonies (Fortelius et al. 1987). However, the colony-specific weight of workers was not measured in 2008, so their colony-specific weight was estimated based on head width data. The head width and dry weight of workers of *F. exsecta* in this population are highly correlated (linear regression: $r^2 = 0.610$, $b = -11.03$, $N = 310$; E. Vitikainen, unpubl. data 2005–2006), so we used head widths of workers from the present study colonies measured for another study (Stephen J. Martin, unpubl. data) to calculate colony-specific averages for worker weight, based on the regression equation $y = 0.610x - 11.03$. The colony-specific worker weights were then used to calculate the total biomass brood produced by the study colonies. The effects of colony inbreeding and colony size on colony productivity and the size of reproductives were tested using GLM. We excluded nonsignificant variables with a stepwise backwards elimination of terms following a usual procedure (Zar 1999). When removing terms did not improve the model, we present the full model. We used log transformation on measurements of weights and head widths, whereas colony size and number of queens were square-root transformed to improve normality of the residuals. The residuals obtained from the GLM were normally distributed in all analyses (All Wilk-Shapiro tests $P > 0.08$). We used the false discovery rate (FDR; Benjamini and Hochberg 1995; Benjamini and Yekutieli 2001) to account for multiple testing. All analyses were done using SPSS 17.0.0 (SPSS Inc.)

Results

INBREEDING, COLONY GENETIC STRUCTURE, AND PRODUCTIVITY

In all colonies, worker genotypes across all loci were consistent with a single queen, which was singly mated in 45 colonies and multiply mated in 11 colonies. The average expected inbreeding coefficient of the workers, F_{IS}, calculated as the relatedness between parents (Liautard and Sundström 2005) was 0.08 ± SE 0.008 (range: −0.3 to 0.51). The average inbreeding based on homozygosity (HL, Aparicio et al. 2006) weighed by the information content of the loci was 0.23 ± 0.016 (mean ± SE; range across colonies 0–0.54). As expected, the two measures of inbreeding were highly correlated (Pearson $r = 0.87$, $P < 0.0001$, $N = 56$). We henceforth use HL as a measure of inbreeding, as this method is not subject to the uncertainties that may arise when parental genotypes are deduced from offspring. All results were qualitatively the same when using relatedness instead.

On average, colonies had 2311 ± 196 adult workers (mean ± SE, $N = 52$) and produced 3483 ± 276 brood items ($N = 39$), 2538 ± 235 of which were reproductives ($N = 39$). The biomass brood produced was 13.19 ± 1.47 g for all brood, and 11.16 ± 1.35 g for reproductive brood ($N = 28$ and $N = 30$, respectively). Colony size and inbreeding were not significantly correlated (Pearson $r = -0.039$, $P = 0.77$, $N = 52$), and colony size affected neither total production (numbers), the number of reproductives produced, nor the number of diploid brood items (Table 1). However, inbred colonies produced significantly less total biomass brood and less reproductive biomass, although there was no significant effect on the total number of brood items produced (Table 1, Fig. 1). In addition, worker efficiency, in terms of reproductive brood produced per worker, decreased significantly with both increased inbreeding and colony size (Table 1, Fig. 1), however, the effect of colony size was not statistically significant after correcting for multiple testing. The fact that workers in larger colonies rear fewer reproductives per capita explains why overall productivity does not depend on colony size.

The within-colony levels of inbreeding did not differ significantly between contemporary gyne and worker brood (paired T-test: $t_{14} = -0.39$, $P = 0.70$, averages 0.27 ± 0.043 and 0.27 ± 0.042, respectively). However, when considering the variation

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Table 1. Summary table of all the GLM-analyses on the effects of inbreeding and colony size on colony productivity and sex ratio

Dependent variable	Main effect	df	*F*	*P*(*F*)	Direction of effect	Dropped variables	R^2	Model *P*
Total biomass (g)	Inbreeding	1, 26	8.870	0.006	−	*P* > 0.2: Island, Colony size	0.254	0.006
Reproductive biomass (g)	Inbreeding	1, 26	13.014	0.001	−	*P* > 0.4: Island, Colony size	0.269	0.003
Reproductive biomass per capita (mg)	Colony size Inbreeding	1, 26 1, 26	5.16 5.30	0.032* 0.030	+ −	*P* > 0.4: Island	0.295	0.011
Total production (numerical)	Island Inbreeding	3, 34 1, 34	2.658 2.832	0.064 0.102		*P* > 0.3: Colony size	0.152	0.065
Diploid brood (numerical)	Colony size Inbreeding	1, 36 1, 36	0,987 2.384	0.327 0.131			0.085	0.203
Number of gynes	Inbreeding	1, 47	11.450	0.001	−	*P* = 0.13: Colony size	0.196	0.001
Number of males	Colony size Inbreeding	1, 36 1, 36	0.031 0.044	0.862 0.834			0.002	0.96
Sex ratio (numerical)	Inbreeding	1, 52	7.617	0.008	−	*P* > 0.3: Island, Colony size Queen mating frequency	0.128	0.008
Proportion of workers of the diploid brood (numerical)	Colony size Inbreeding	1, 44 1, 44	2.157 5.294	0.149 0.026	 −		0.169	0.017

****P*-values marked with an asterisk were not significant after correcting for false discovery rate (FDR, Benjamini and Hochberg 1995).**

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across colonies, inbred colonies produced a lower fraction of gynes among diploid brood (Table 1). Furthermore, when gyne and male brood were considered separately, inbred colonies produced fewer gynes, whereas the number of males was unaffected (Table 1, Fig. 2). Consequently, sex ratios were more male-biased in inbred colonies (Table 1). Once again colony size had no significant effect on either the sex ratio or the number of gynes or males produced (Table 1, Fig. 2).

SIZE OF REPRODUCTIVES AT ECLOSION AND AT MATURITY

Gyne size, measured as head width, differed significantly among colonies but not within colonies between callow (1.73 ± 0.003 mm, $N = 187$) and mature gynes (1.73 ± 0.003 mm, $N = 233$; overall average 1.73 ± 0.005 mm, $N = 391$; Fig. 3A; Table 2). Male size also differed significantly among colonies, but not within colonies between callow (1.51 ± 0.012 mm, $N = 89$) and mature males (1.52 ± 0.005 mm, $N = 405$; overall average 1.52 ± 0.005 mm, $N = 494$; Fig. 3A; Table 2). Hence, our samples of immature and mature females and males comprise comparable random sets of the reproductives produced in each colony. Male size was significantly more variable than gyne size (Levene's statistic for equality of variances = 169.04, df = 1, 950, $P < 0.001$).

Gynes gained on average 0.61 ± 0.07 mg (range: 0.1–1.33 mg, $N = 21$) between eclosion and maturation; consequently, mature gynes were significantly heavier than callow ones (4.89 ± 0.034 mg, $N = 238$, and 4.22 ± 0.04 mg, $N = 186$, respectively; Table 2; Fig. 3B). By contrast, mature males were not significantly heavier than callow ones (3.88 ± 0.037 mg, $N = 432$ and 3.81 ± 0.075 mg, $N = 72$, respectively; Table 2; Fig. 3B). Gyne size was not correlated with any of the tested colony traits (Table 3), but we included gyne head width in the model to account for differences in relative weight in response to colony traits.

Gyne weight at maturity increased with gyne head width, so we included head width to control for variation in gyne size when appropriate (Table 3). Neither gyne weight at maturity nor gyne weight gain during maturation, were affected by inbreeding,

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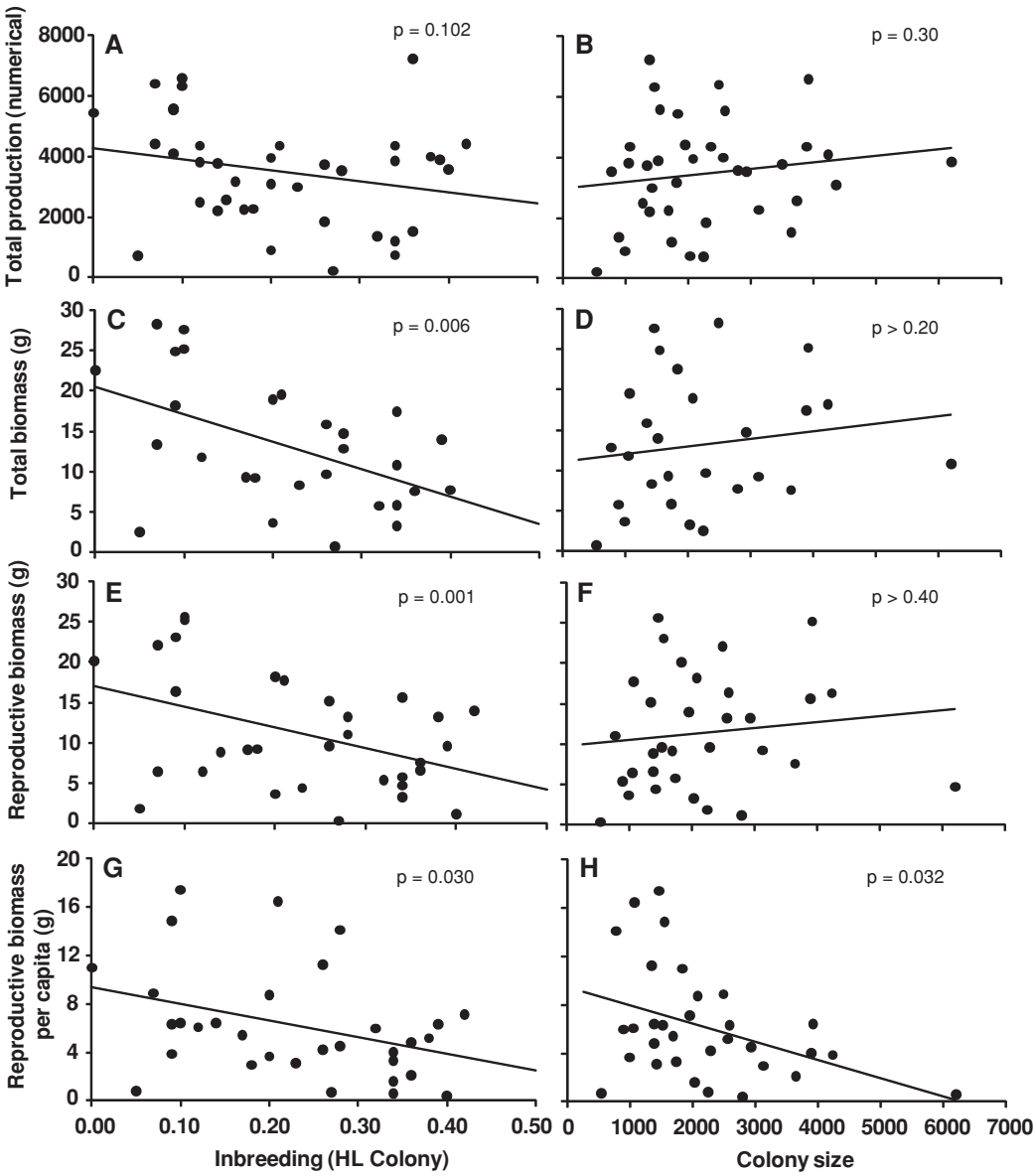

Figure 1. Effects of inbreeding and colony size on the total numbers of brood (A and B), total biomass (C and D) reproductive biomass (E and F), and per capita productivity (G and H), and the associated *P*-value from the GLM-analyses (See Table 1).

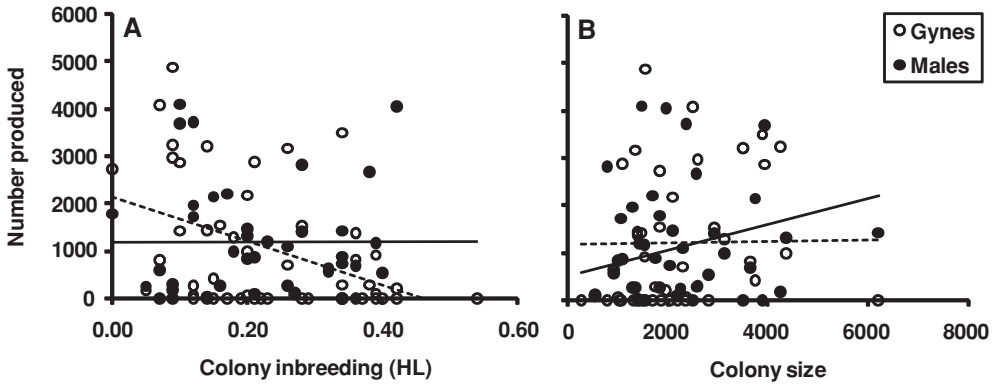

Figure 2. Effects of colony inbreeding (A) and colony size (B) on numbers of males (solid lines) and gynes (dotted lines).

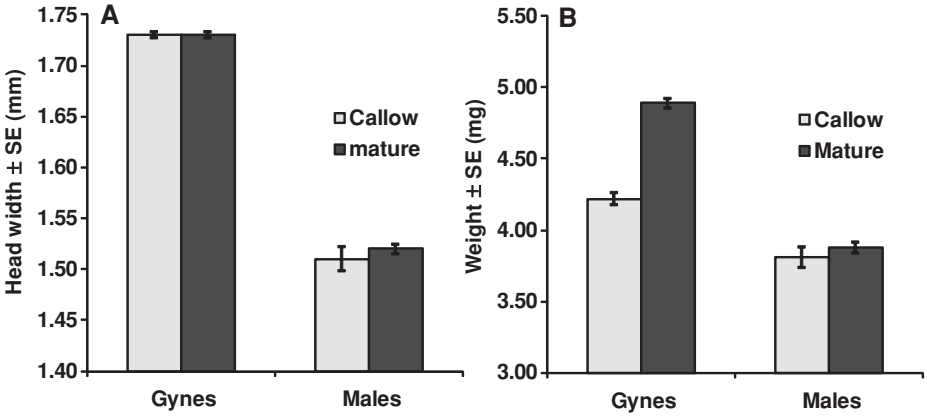

Figure 3. Average head width (A) and weight (B) of callow and mature gynes and males.

but both increased significantly with colony size (Table 3). There was, however, a significant interaction between colony size and the number of gynes produced on gyne weight at maturity (Table 3). As a result, the net effect of colony size on gyne weight at maturity is nil, because large colonies produce marginally, but not significantly, more gynes than small colonies (Table 1), and colonies that produce more gynes produce marginally lighter ones (Table 3, Fig 4). Colony averages of male weight and head width were highly correlated (Pearson R = 0.84, $P < 0.0001$, $N = 47$), so we only used the effect on male weight as a predictor in the model (analyses using head width give qualitatively similar results). Males from highly inbred colonies were significantly smaller than those from less inbred colonies, but neither colony size nor the number of males produced in the colony was associated with average male weight (Table 3, Fig. 4).

Discussion

In this study, we tested whether inbreeding in the worker caste affects patterns of reproductive investment at the colony level, as well as fitness-related traits of reproductive offspring. The results show that worker inbreeding impairs colony performance with cascading effects on sexual production, sex allocation, reproductive allocation, and the weight of offspring males, but not gynes. These changes in resource allocation nonetheless follow adaptive patterns predicted by theory, as colonies adjust sex ratio, reproductive allocation, and offspring size in accordance with optimality predictions when trade-offs between offspring number and quality prevail (Smith and Fretwell 1974).

COLONY PERFORMANCE AND INBREEDING

Both the total biomass of brood produced and the biomass of reproductive brood decreased when the level of colony inbreeding increased, yet neither the total number of brood nor the number of diploid brood declined with inbreeding. This implies that the egg-laying rate of the queens remained unaffected by worker inbreeding, and that the mortality of inbred brood at an early life stage is not causing the decline in productivity. Limitations in the egg-laying capacity of the colony queen may, however, prevent productivities beyond a certain limit, leading to the proportionately lower productivity observed in large colonies. Indeed, inefficiency in work performance in large colonies has been suggested to contribute to lower per capita productivity (Michener 1964; Oster and Wilson 1978; Kolmes 1986; Walin et al. 2001). Nonetheless, inbred colonies were less productive in terms of biomass of brood produced, irrespective of colony size, which implies an additional decline in colony performance due to inbreeding. A reduction in productivity of inbred colonies could

Table 2. The effect of colony of origin and maturation stage on the head width and weight of reproductives, nested ANOVA with colonies nested under maturation stage (callow or mature).

		Gynes			Males		
	Factor	df	*F*	*P*	df	*F*	*P*
Head width:	Maturity	1, 76.2	0.06	0.81	1, 41.9	0.69	0.41
	Colony	38, 240	2.09	<0.0001	40, 173	14.27	<0.0001
Weight:	Maturity	1, 45.9	15.66	<0.0001	1, 42.3	0.69	0.41
	Colony	38, 239	8.96	<0.0001	40, 172	12.77	<0.0001
	Head width	1, 239	1.97	0.161	1, 172	4.33	0.039

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Table 3. Summary of GLM analyses of colony characteristics on average weight of gynes and males

Dependent	Main effects	df	F	$P(F)$	Direction of effect	Dropped variables	Model R^2	Model P
Average size of gynes (head width)	Colony size	1, 19	0.789	0.385			0.083	0.784
	Inbreeding	1, 19	0.203	0.509				
	Number of gynes	1, 19	0.434	0.518				
	Colony size *N gynes	1, 19	0.957	0.340				
Average weight of mature gynes (mg)	Head width	1, 19	8.537	0.009	+	$P > 0.6$:Inbreeding	0.461	0.015
	Colony size	1, 19	7.971	0.011	+			
	Number of gynes	1, 19	4.150	0.056	−			
	Colony size *N gynes	1, 19	7.859	0.011				
Gyne weight gain during maturation (mg)	Colony size	1, 16	8.251	0.011	+	$P > 0.6$:Inbreeding, Number of gynes	0.34	0.011
Average male weight (mg)	Colony size	1, 42	2.709	0.107		$P > 0.4$: Island, Number of males	0.148	0.035
	Inbreeding	1, 42	5.904	0.019	−			

ensue if diploid eggs intended to become gynes instead developed into diploid males, and if such diploid males were destroyed by the workers before pupation. This is known to occur in inbred colonies of the honey bee *Apis mellifera* (Woyke 1963). If so, more inbred colonies should produce fewer gynes, which indeed was the case. However, the total number of diploid brood produced did not change with inbreeding, which implies that the presence of diploid male brood and its subsequent removal cannot account for the observed decline in gyne production.

The decrease in biomass productivity with increased inbreeding may be the consequence of energetic costs incurred by inbred workers, reducing the resources available for rearing offspring. Indeed, inbreeding depression has been considered a form of genetic stress, such that inbred individuals have lower metabolic

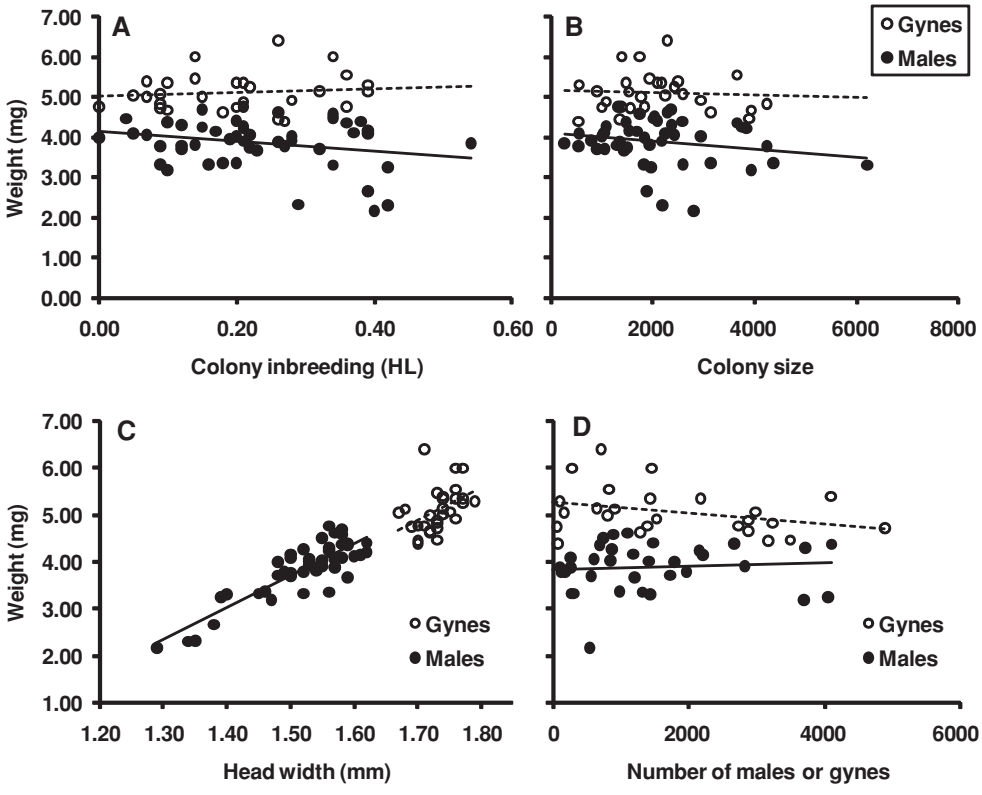

Figure 4. Correlation between colony averages for male and gyne weight and colony inbreeding (A), colony size (B), head width (C) and the number of same-sex reproductives (D)

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efficiency (Myrand et al. 2002; Kristensen 2006). In addition, the performance of colonies with inbred workers may be compromised if workers have higher parasite loads themselves, or are less able to fend off parasites and pathogens (Liersch and Schmid-Hempel 1998; Baer and Schmid-Hempel 1999; Gerloff et al. 2003; Tarpy 2003; Ugelvig et al. 2010; Vitikainen and Sundström 2011) More generally, higher genetic diversity of workers has been associated with improved foraging efficiency and colony growth (Oldroyd et al. 1992, 1993; Cole and Wiernasz 1999), improved homeostasis (Jones et al. 2007; Oldroyd and Fewell 2007), and signaling performance (Duong and Schneider 2008). Hence increased genetic diversity may improve the ability of workers to adjust to variation in resource levels, temperature, and other environmental variables (Oldroyd and Fewell 2008). Thus, inbreeding may have a wide range of negative effects on colony performance.

SIZE OF REPRODUCTIVES

Males produced by inbred colonies were lighter and hence smaller than those produced by less-inbred colonies, whereas no such effect was found for gynes at maturity. Neither colony size nor the number of males or gynes produced had an effect on male or gyne size, respectively. Although large colonies did not produce more gynes than small ones, the gynes produced by large colonies gained significantly more weight between eclosion and maturity. This suggests that the number of gynes produced was traded-off against gyne size and weight, and that large, presumably well resourced, colonies invest in heavier gynes, rather than greater numbers of them. No such trade-off is evident for males.

The effect of inbreeding on male size shows that the effects are mediated by factors related to worker functions, as haploid males cannot be inbred like their sisters. Although males used in this study were not genotyped, the effect of diploid males on the average male size is negligible, given that the prevalence of diploid males is only 5% in this population (E. Vitikainen, unpubl. data). Furthermore, diploid males are generally larger, not smaller than haploid ones, (e.g., the ants *Solenopsis invicta,* Ross and Fletcher 1985 and *Lasius sakagamii,* Yamauchi et al. 2001; the turnip sawfly *Athalia rosae*, Naito and Suzuki 1991). Only one of our study colonies produced diploid males, which were very close to the population average in size. Therefore, the most likely explanation for the reduced size in males is that the effects of inbreeding are mediated through the inbred workers of the colonies, not by the genetic makeup of the males themselves.

The extent to which decreased male size affects their fitness is unknown in this species, but in insects in general, a larger body size confers fitness benefits (Hood and Tschinkel 1990; Stockhoff 1991; Roff 1992; Ohgushi 1996; Raubenheimer and Simpson 1997; Nylin and Gotthard 1998; Blanckenhorn 2000; Reim et al. 2006). Ant males, however, only live to mate during their nuptial flight, and intrasexual selection is likely to be relaxed in such species (Boomsma et al. 2005; but see Abell et al. 1999). Hence, male fitness in ants may be less dependent on the size of the individual, at least in species in which males are unlikely to mate more than once. Indeed, consistent with relaxed selection, male size was much more variable than gyne size. For gynes, size is likely to be crucial for survival through colony founding, most of which is carried out using body reserves of fat and glycogen (Keller and Passera 1989; Fjerdingstad and Keller 2004). The smaller size variation in gynes than males may also be due to lower plasticity in the size of gynes due to the caste determination mechanism, or selection may act more strongly on gyne size, thus maintaining a minimum size.

INBREEDING AND SEX ALLOCATION

Colonies with inbred workers produced similar numbers of males as noninbred ones, but fewer gynes. As the total number of diploid brood did not change with inbreeding, fewer gynes were produced because fewer of the diploid larvae entered the gyne development pathway. As a result, colony sex ratios were more male-biased in inbred colonies, mainly owing to this change in caste fate of diploid brood. Such a change in developmental trajectory could be mediated through intrinsic processes, for example, if homozygosity directly affects caste fate, for instance through decreased production of juvenile hormone by the larvae (Wheeler 1986). This is an unlikely explanation, because we found no evidence for within-colony differences in homozygosity between worker and contemporary gyne brood. Indeed such a difference would not be expected given random segregation across a large number of chromosomes ($2N = 52$; Hauschtek-Jungen and Jungen 1976). This suggests that colony performance may impinge on caste allocation among diploid brood in colonies composed of inbred workers, irrespective of homozygosity of larvae themselves. As gyne larvae are bigger than those developing into workers, the most obvious way by which ant workers affect caste determination of diploid larvae is by providing them food for growth. If inbred workers are less proficient, fewer larvae may gain the necessary resources to develop into gynes.

The effect of inbreeding in shifting colony sex ratios toward male bias also has implications for optimal sex allocation. An earlier study on the same population found that sex allocation followed the predictions from split sex ratio theory, indicating worker control of sex ratios (Boomsma and Grafen 1990, 1991), with colonies headed by a singly mated queen specializing in gyne production and colonies headed by a multiply mated queen specializing in male production (Sundström et al. 1996; Chapuisat et al. 1997). In the present study, mating frequency did not affect the colony sex-ratio, suggesting that the colonies produce a suboptimal sex ratio from the workers' inclusive fitness perspective. Inbreeding may also impact population-wide sex ratios and so act

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to shift these in a direction disfavored by workers but favored by resident queens (Trivers and Hare 1976).

CONCLUSIONS

Our results show that the size of reproductives is traded off against numbers, differently for females and males, such that individuals of the sex whose fitness depends less on size and weight are produced in similar numbers, but the individuals are smaller, whereas the sex whose fitness is contingent on adequate parental investment maintains size and weight, but fewer are produced. This pattern is consistent with the expectations under optimal resource allocation in conjunction with resource limitation, when the reproductive success of male and female offspring depends to different degrees on the parental investment they receive (Smith and Fretwell 1974). Given that ant workers have control over the size of reproductives they rear (Fjerdingstad 2005), impaired worker performance owing to inbreeding can nonetheless to some extent be salvaged through a redirection of allocation.

Our results also show that inbreeding decreases both the production of sexual females and the size of males in colonies with an inbred worker force and that these effects are likely to be mediated through the workers, rather than the genetic make-up of the offspring themselves. If so, a single mechanism, limited growth rate of larvae in colonies with inbred workers, could explain both outcomes if reduced growth affects caste determination in female larvae and adult size of males, respectively. Increased genetic stress mediated by inbreeding could account for a reduction in growth rate through the workers via impaired colony performance. The result is a direct decline in colony fitness through lower reproductive output, but there is also an indirect effect because the gynes produced by the colony are as inbred as their sister workers. Indeed, inbred young gynes have upregulated immune defense (Vitikainen and Sundström 2011), which may be costly in itself, or reflect compromised energy allocation owing to inbreeding. In any case, *F. exsecta* colonies headed by inbred queens have a shorter life span than less-inbred ones (Haag-Liautard et al. 2009).

To date only a few studies have considered the effects of inbreeding in tending individuals on the growth and survival of their young (e.g., Cordero et al. 2004; Ortego et al. 2010), but to our knowledge no studies so far consider these effects in social animals. Taken together, our results show that inbreeding in the worker caste can have profound consequences for fitness in social animals, and that these effects can be mediated by colony-level processes with cascading effects on population-level sex ratios, colony-level allocation patterns, and individual-level fitness-related traits.

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